3

Studies on the Endocrine Glands of the Salmonoid Fish, the Ayu, *Plecoglossus altivelis* Temminck & Schlegel. V. Seasonal Changes in the Endocrines of the Land-locked Form, the Koayu

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(Plates I-III; Text-figure 1)

IT IS WELL KNOWN that there are several differences in the shape of the body and mode of life of the ordinary Ayu, *Plecoglossus altivelis*, found in most rivers of Japan, and the dwarf Koayu[1] which is landlocked in lakes[2]. The Koayu is smaller than the Ayu, owing to the smaller amount of food in its environment. Moreover, Koayu that have survived after spawning (the so-called Otu-nen Koayu) are more numerous than postspawning Ayu. Since details of the life history and ecology of the Koayu are not fully known at present, a comparison of the function and a correlation of the secretory activity of endocrine glands of the Koayu and Ayu would be of value, particularly since the situation in the Ayu has been clarified by the senior author (Honma, 1959 a, b.; 1960, 1961). For the purpose of such a comparison, seasonal changes in the thyroid, pituitary, adrenal cortical tissue and gonads of Koayu have been observed histologically.

MATERIALS AND METHODS

The fish used in this study were collected monthly from Lake Biwa, the largest lake in Japan, with the cooperation of the Shiga Prefectural Fisheries Experimental Station. The period of collection extended from March, 1960, to September, 1961 (Table I). Five females and five males were selected for examination from each monthly collection, and preserved in Bouin's fixative. The glands were removed and embedded in paraffin, cut serially at 8 to 10μ and stained with Delafield's hematoxylin-eosin, Heidenhain's hematoxylin-light green, Heidenhain's azan triple stain, periodic acid-Shiff technique as modified by Wilson & Egrin (1954), Gomori's chrome alum hematoxylin-phloxine, and Halmi's paraldehyde fuchsin-orange G and light green.

OBSERVATIONS

Thyroid.—In order to follow thyroid function, changes in the height of the follicular epithelial cells of the thyroid gland throughout the lifespan of the fish were measured and plotted (Text-fig. 1). The thyroid follicles of juvenile fish (the so-called Hiuwo, which has a transparent body) from November to the following March are in a condition of inactivity (Pl. I, fig. 1). The follicles scattered in the neighborhood of afferent branchial arteries and anterior ventral aorta are few in number, the epithelia consist of cubical cells, and the colloid within the follicular lumina is stained moderately with eosin. The thyroid of young fish during their upstream migration period (April) indicates hyperactivity, and the epithelia of the follicles are composed of high columnar cells (Pl. I, fig. 2). In the growing period (May to June) the epithelium rapidly decreases in height, and the gland shows features of hypofunction. The highest level, however, is reached in the prespawning season (July to August) (Pl. I, fig. 3); at that time the fish which have grown in the lake ascend the rivers to seek spawning places. Re-

[1]Ko means small or dwarf in Japanese.

[2]Lakes Biwa-Ko, Ikeda-Ko, Unagi-Ike, Toyoda-Ko, Ono-Ko and Sai-Ko.

TABLE I. *Plecoglossus altivelis* TEMMINCK & SCHLEGEL FROM LAKE BIWA, JAPAN

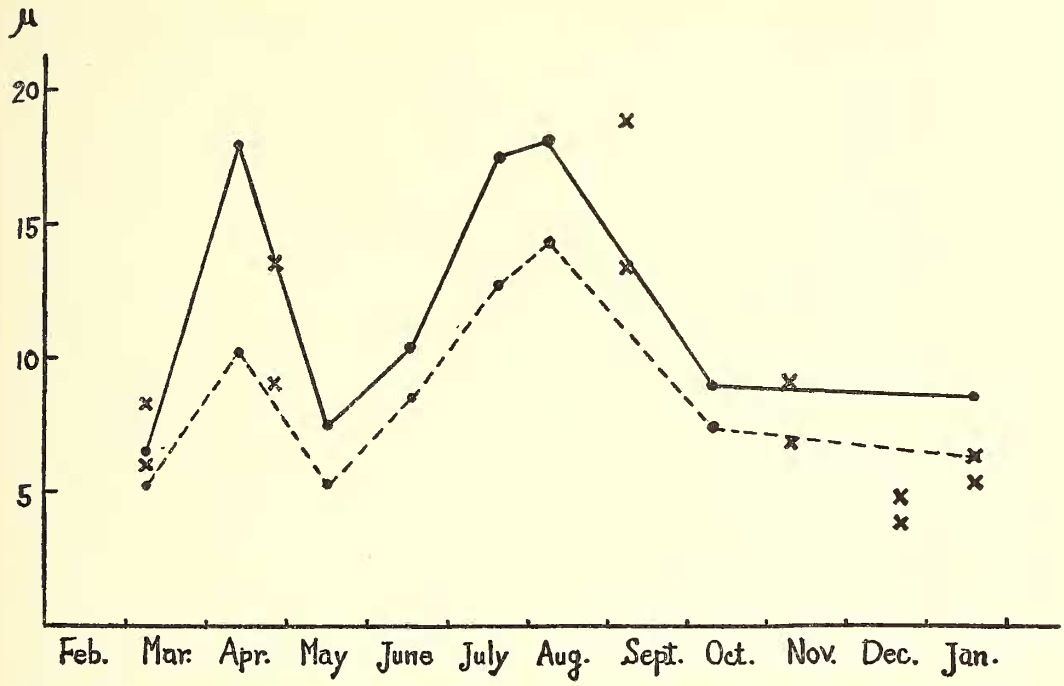
Date of collection		Number of Fish		Locality	Ontogenetic Stage
		♀	♂		
March 7, 1960		5	5	Lakeside, Nishiasai-mura	Young lake fish
April 12, 1960		5	5	Mouth of Hino-gawa river	Young fish at time of ascent of river
May 16, 1960		5	5	Lakeside, Nishiasai-mura	Immature lake fish
June 16, 1960		5	5	Lakeside, Asai-mura	Lake fish during growth period
July 19, 1960		5	5	Ane-gawa river	Mature fish during upstream migration
August 8, 1960		5	5	Oh-gawa river	Mature fish during upstream migration
September 7, 1961		5	5	Mouth of Inugami-gawa river	Adult fish at spawning period
October 10, 1960		5	5	Amano-gawa river	Fish immediately after spawning
November 8, 1960		5	5	Lakeside, Hikone City	Juvenile lake fish (so-called Hiuwo, *i.e.*, Shirasu larvae)
December 20, 1960		5	5	Lakeside, Maibara-machi	Juvenile lake fish
January 17, 1961	Adult	5	5	Lakeside, Ado-gawa-machi	So-called Otu-nen Koayu
	Underyearling	5	5		Juvenile lake fish
February, 1961		0	0		
March 7, 1961		5	5	Lakeside, Nishiasai-mura	Young lake fish
April 25, 1961		5	5	Mouth of Yogo-gawa river	Young fish at time of ascent of river

markably, the nuclei are now situated at the center or the apical end of the high columnar cells, and the volume of colloid is very scanty. Nearly identical histological figures are encountered in specimens obtained during the breeding season (September) (Pl. I, fig. 4), and there is a noticeable exhaustion of colloid. After the completion of spawning, the thyroid returns to a state of low activity and most of the fish die (Pl. I, fig. 5). The gland of the spent fish and Otu-nen Koayu pass into a typical hypofunctioning state; the follicle is surrounded by flat epithelium and the lumen contains much colloid (Pl. I, fig. 6).

Pituitary Gland.—Changes in the external appearance of the pituitary gland of the Koayu throughout its life-span coincide well with that of the ordinary Ayu. Although the pituitary of juvenile fish is a longish ovoid, there is a notable ventral projection of the gland in young fish. During the maturation period the gland swells and transforms into a hemispherical body. The shape of the gland of Otu-nen Koayu, however, is somewhat protuberant.

Until the prespawning season, the meso-adenohypophysis is composed chiefly of acidophils. At the time of onset of vitellogenesis, *i. e.*, the beginning of yolk vesicle formation in ovarian eggs, and of development of spermatocytes into spermatids (August), the PAS- and AF-positive polygonal cyanophils gradually increase in number, forming islands. Maximal development of cyanophils is reached in the breeding season. At this stage, the cyanophils located in the ventral region of the gland form many interconnected peninsulae. Many lacunae, varying in size, are found in the glandular portion of the pituitary, particularly in the pro-adenohypophysis (Pl. II, fig. 1). The neurohypophysis in the breeding season has a small quantity of CHP- and AF-positive neurosecretory material, but it is densely filled with such materials in the postspawning spent fish. Moreover, the gland of the spent fish has a degenerate appearance, accompanying the decrease in the number of cyanophils and the remarkable development of lacunae.

Adrenal Cortical Tissue.—The amount of adrenal cortical tissue in juvenile fish is small, there being merely two or three layers of the tissue surrounding the wall of the veins and venules in the neck region of the slender head kidney



TEXT-FIG. 1. Follicular epithelial cell height of thyroid gland throughout the life-span of Lake Biwa Koayu. Solid line: cell height, longer axis. Broken line: cell height, shorter axis.
Dots represent specimens collected in 1960 (Oct., spent fish; Jan., Otu-nen Koayu).
Crosses represent specimens collected in 1961 (Nov. to Jan., Hiuwo).

(Pl. II, fig. 6). Cubical cells with rather large round nuclei form the cord. Invasion of the tissue into the adjacent lymphoid tissue takes place as the gonad matures (Pl. II, figs. 2, 7). The tissue increase occurs through cell division, and the increase in number of cells is remarkable. In the breeding season, the tissue becomes a large hyperplastic mass with sinusoids running through it (Pl. II, fig. 3). The postspawning Koayu, however, shows no marked vascularity or severe internal hemorrhage such as has been described in the ordinary Ayu (Pl. II, figs. 4, 5, 8).

Gonads.—As the authors intend to publish details of the seasonal changes in the gonads of Koayu elsewhere, in the present paper they wish only to mention briefly the process of development of germ cells and the appearance of spent gonads.

The gonad of juvenile fish (Hiuwo) taken in November is in the form of a ridge originating from the dorsal fold of the splanchnic mesoderm, and the formation of primordial germ cells takes place in that membrane (Pl. III, fig. 1). Sexual differentiation is completed in the following January, at which time differences between the ovary and testis become histologically distinct. The ovarian eggs pass through the state of yolkless, chromatin nucleolus and perinucleolus stages by turns, from January to July (Pl. III, fig. 2). Vitellogenesis begins in the prespawning season (August) and the eggs proceed from yolk vesicle to yolk globule stages. In the breeding season (September), many of them rapidly reach a ripe state, and the micropyle and thick adhesive membrane surrounding the animal pole of the egg complete their development (Pl. III, fig. 3). However, the developmental stages of the ovarian eggs contained in a single Koayu ovary are not synchronous. After ovulation (October), atretic eggs in the process of the formation of a type of corpus luteum, and ovulation scars are both found in the spent ovary, but a considerable number of eggs in the yolk vesicle and perinucleolus stages are also present (Pl. III, figs. 4, 5). Details of the course of regression of the atretic eggs of Koayu are omitted here, because the picture coincides well with that in the Ayu. On the other hand, in the ovigerous folds of the spent ovary of Otu-nen Koayu, several groups of young oocytes at the chromatin nucleolus stage have been encountered. These are contained in nests which may have originated from those of residual oogonia (Pl. III, fig. 6).

During January to July, the spermatogonia in the testis are in the stages of growth and multiplication. Growth and further maturation division of spermatocytes is detectable in the prespawning season (August), and the formation of spermatids takes place rapidly. Eventually, early in September, the process of transformation of spermatids into spermatozoa becomes recognizable (Pl. III, fig. 7). The cysts filled with functional sperm burst one by one, and the sperm are ejected into the sperm duct. As a result, a large chamber is found in the testis (Pl. III, fig. 8). In the cystic wall of the spent testis there appears a layer of cells which might be newly formed spermatogonia.

Discussion

Recently, the role of the thyroid gland in the development of the platyfish has been elucided by Baker-Cohen (1961). She concluded that the thyroid plays a significant role in the growth, sexual maturation and liver metabolism of fish. A direct or adjunctive osmoregulatory role of the thyroid gland in the starry flounder has been suggested by Hickman (1959). In his previous paper, the senior author considered that the thyroid function of the Ayu is correlated to some extent with the osmotic regulation of salt-water balance, because the peak thyroid activity is found at the time of entering the river from the sea (Honma, 1959 a). Hoar *et al.* (1952, 1955) reported a correlation between thyroid activity and the locomotion of salmon and goldfish. It has been shown that a continued high level of thyroid activity may initiate and sustain the long migration of both the sexually mature and immature cod in the Barents Sea (Woodhead, 1959 a, b). Therefore, a possible relation between thyroid function and the upstream migratory behavior or active locomotion of the Koayu in Lake Biwa may be intimated from the present investigation, where there was no rapid change in osmotic conditions. It is difficult to decide whether or not the peak of thyroid activity before and during the breeding season of the Koayu is related to sexual development, even though increased thyroid hormone demand in fish prior to or during spawning is well known (Olivereau, 1954, Pickford & Atz, 1957). The direct significance of the thyroid hormone itself to the sexual cycle of fish has not been yet elucidated.

Although it is difficult to make a clearcut distinction between, as well as a separation of, the thyrotrophs and gonadotrophs in the pituitary gland of fish, the so-called basophil (cyanophil) islands, located in the ventral region of the meso-adenohypophysis, are well demonstrated with azan triple-, PAS- and AF-stain. Conspicuous cyclic changes correlated with those occurring in the gonads have been reported by many investigators, *e. g.*, Bretschneider & Duyvené de Wit (1947), Scruggs (1951), Sokol (1961) and Robertson & Wexler (1962, a, b). Similar changes in the pituitary of the Ayu have been observed by the senior author (Honma, 1959 a, b). Since the time of appearance of the basophil islands coincides well with the commencement of ovarian vitellogenesis and of spermatid formation, the cyanophils in the ventral region of the gland of Koayu seem to be the gonadotropic cells. The pituitary degeneration that occurs in the postspawning rainbow trout and Pacific salmon has been well described by Robertson & Wexler (1962 a, b), and the present study has also demonstrated it in the Koayu.

That the adrenal cortical tissue of lampreys, salmon and Ayu undergoes a conspicuous hyperplasia in correlation with the maturation of gonads has been demonstrated by Sterba (1955), Robertson (1958), Robertson & Wexler (1959, 1960), Honma (1960) and Oguri (1960). Although hyperplasia also occurs in the cortical tissue of the Koayu up to the breeding season, there is no intense vascularization, as was reported in the tissue of the Atlantic salmon at the time of smoltification (Olivereau, 1960) nor internal hemorrhage as is found in the Ayu (Honma, 1960). A marked increase in the plasma 17-hydroxycorticosteroids of the king salmon accompanying sexual maturation and spawning was reported by Hane & Robertson (1959) and Robertson *et al.* (1961). Therefore, it seems likely that adrenal hyperplasia in fish is an indication of a condition of hyperadrenocorticism. The summer adrenal hyperplasia of the Koayu seems to be more related to the severe derangement of metabolism owing to the development of the gonad than to the energy demand of upstream migration.

The seasonal histological changes in the ovary and testis and the process of the formation of corpora lutea in the atretic eggs of both shark-like and bony fishes have been described by many investigators, *e. g.*, Beach (1959), Bretschneider & Duyvené de Wit (1947), Gokhale (1957), Hisaw & Hisaw (1959), Honma (1961) and Stolk (1951, 1957). In a previous paper (Honma, 1961), the senior author stated that he could not recognize an ultimate corpus luteum structure (*d*-phase) comparable with the one described by Dutch workers, and Polder (1961) has failed to demonstrate it in the herring. The present study has also failed to find it in the Koayu.

Gametogenesis in the Koayu occurs about a month earlier than in the Ayu. The mode of development of the ovarian eggs of the Koayu seems to be referable to partial synchronism, since the development of young oocytes originating from the residual oogonia in the ovigerous fold takes place during and after the breeding season. It is not yet determined, however, whether or not these eggs are ejected after maturation. Similarly, the fate of the spermatogonia regenerated in the cyst wall of the spent testis is questionable, because male Koayu that have survived the winter have not yet been obtained by the present authors.

Whether or not the fish can survive after spawning as Otu-nen Ayu (Ayu and Koayu) probably depends upon the degree of exhaustion of the endocrine glands—in particular the adrenal cortical tissue—and the quantities of atretic and unovulated, partially ripe eggs contained in the spent ovary as a partial source of nutrition.

Summary

The changes occurring in the thyroid, pituitary, adrenal cortical tissue and gonads of the land-locked salmonoid fish, *Plecoglossus altivelis* T. & S., (Koayu) throughout its life-span were examined histologically and compared with those of the anadromous form of the same species (Ayu). The Koayu were collected from Lake Biwa, Japan.

1. Two peaks of thyroid activity were plotted in the spring and summer runners, compared with only one peak of gland activity in the Ayu. A possible relation between thyroid function and the upstream migratory behavior or active locomotion of the fish is suggested.

2. The time of appearance of the so-called basophil islands in the ventral region of the pituitary gland coincides well with the onset of ovarian vitellogenesis and spermatid formation. These aniline blue-, PAS- and AF-positive, polygonal cyanophils seem to be the source of gonad-stimulating hormone. A decrease in the number of cyanophils was detected in spent fish.

3. A marked increase in the amount of adrenal cortical tissue takes place following the maturation of the gonads. There is, however, no extreme vascularity nor internal hemorrhage such as is found in the hyperplastic tissue of the Ayu.

4. Gametogenesis occurs about one month earlier than in the Ayu. Development of the ovarian eggs of the Koayu might be classified as partial synchronism. Whether young oocytes originate from the residual oogonia in the ovigerous folds of spent ovary and whether spermatogonia regenerate in the cyst wall of the spent testis is still uncertain.

The process of formation of corpora lutea in the atretic eggs and the absence of an ultimate corpus luteum structure coincides with what has been found in the Ayu.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Thyroid follicles of juvenile fish (Hiuwo) in a condition of hypoactivity. ×250

Fig. 2. Thyroid follicles of young fish with high columnar epithelial cells, during upstream migration. ×250

Fig. 3. Thyroid follicles of prespawning fish in a condition of hyperactivity, at the time of ascending the river. ×250

Fig. 4. Thyroid follicles of spawning fish showing noticeable exhaustion of colloid. ×250

Fig. 5. Thyroid follicles of postspawning fish returned to a state of low activity. ×250

Fig. 6. Thyroid follicles of Otu-nen Koayu which have survived after spawning, consisting of very flat epithelium and a rich supply of colloid. ×250

Plate II

Fig. 1. Pituitary gland of spawning fish in the beginning of condition of degeneration accompanying developing lacunae. ×100

Fig. 2. Adrenal cortical tissue of prespawning fish accompanying the maturation of the gonad. ×150

Fig. 3. Adrenal cortical tissue of adult fish in the breeding season. ×150

Fig. 4. Adrenal cortical tissue of postspawning fish showing no marked vascularity. ×150

Fig. 5. Adrenal cortical tissue of Otu-nen Koayu that have endured the winter. ×150.

Fig. 6. Higher power view of the adrenal cortical cells of juvenile fish (Hiuwo). ×400

Fig. 7. Higher power view of the adrenal cortical cells of young fish at the time of entrance to the river. ×400

Fig. 8. Higher power view of the adrenal cortical cells of spent fish (Otu-nen Koayu). ×400

Plate III

Fig. 1. Primordial germ cells growing in ridge of splanchnic mesoderm of juvenile fish (Hiuwo). ×200

Fig. 2. Young oocytes corresponding to perinucleolus stage in the ovary of immature fish. ×50

Fig. 3. Two mature oocytes with thick adhesive membrane and oolemma. A micropyle and the follicular cells can be recognized. ×100

Fig. 4. Ovary of postspawning fish showing ovulation scars, atretic follicles, and normally appearing oocytes. ×50

Fig. 5. Corpus luteum in *c*-phase in a spent ovary. ×50

Fig. 6. A mass of young oocytes corresponding to the chromatin nucleolus stage in the ovigerous fold of a Otu-nen Koayu. ×200

Fig. 7. Spermatozoa in the testis of adult fish at time of reproduction. ×200

Fig. 8. Testis of spent fish showing vacant cysts. ×200